Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) in recirculated tank production

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Abstract

The efficacy of replacing fishmeal with petfood-grade poultry by-product (PBM) on an ideal protein basis in commercial diets for hybrid striped bass (HSB) was evaluated under production conditions in tank culture. A generic production diet (GEN) for HSB was formulated to contain 45% protein, 12% lipid and 3.7 kcal/kg. Protein in the generic diet was supplied by a mix of animal and plant sources typically used by the industry that included more than 20% select menhaden fishmeal and less than 10% PBM. A positive control diet (GEN+AA) was formulated by supplementing the generic diet with feed-grade Met and Lys to match the level of those amino acids in HSB muscle at 40% digestible protein. Substitution diets were formulated by replacing 35% or 70% of fishmeal in the GEN diet with PBM on a digestible protein basis and then supplementing with Met and Lys (designated 35PBM and 70PBM, respectively) as needed to maintain concentrations of Met and Lys equal to those in the GEN+AA diet. Diet formulation and extrusion were conducted by a commercial mill. Fish were stocked (87 g average initial weight) in three replicated production-scale recirculating culture systems. Diets were initially fed at 4% body weight·day^{-1} divided into morning and evening feedings and gradually decreased to 1.5% body weight·day^{-1} during the 24-week trial. The availability of indispensable amino acids (IAA) in the commercial test diets were determined in a separate trial. All test diets were replete with respect to published requirements of hybrid striped bass; however, available levels of Arg and Thr were first- and second-limiting, respectively, and His was third-limiting, in the replacement diets when compared to the IAA profile of hybrid striped bass muscle.

Diet composition significantly ($P<0.05$) influenced final weight, weight gain, yield, hepatosomatic index (HSI) and intraperitoneal fat (IPF) ratio, but did not significantly alter feed conversion and muscle ratio. Generally, fish fed the 35% replacement diet (35PBM) performed as well as fish fed the generic diet, whereas fish fed the 70PBM diet not. Fish fed the supplemented generic diet (GEN+AA) outperformed fish fed the other test diets. Results clearly demonstrate that formulation on an available amino acid basis can significantly improve the performance of current diets for HSB and that petfood-grade poultry by-product can successfully provide nearly half the protein in commercial HSB diets when substituted for fishmeal on an available amino acid basis.

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1. Introduction

Data regarding amino acid availability in feedstuffs for hybrid striped bass now make it possible to formulate diets on an amino acid availability, rather than gross nutrient digestibility, basis (Gaylord et al., 2004). By-products of the poultry processing industry such as blood meal, feather meal and poultry by-product meal (PBM) are high in protein and contain favorable profiles of indispensable amino acids (IAA) for fish production (Tacon, 1993). Commercial diets for hybrid striped bass already contain some PBM in place of fishmeal; however, inclusion rates are limited to avoid the negative effects observed in other fish when higher inclusion levels have been attempted (David Burris, Burris Aquaculture and Specialty Feeds, Franklinton, LA, USA, personal communication).

One limitation to incorporating substantial levels of PBM in carnivorous fish diets proceeds from factors related to product source and processing. It is well established that source and processing conditions can radically affect specific amino acid availability in rendered products (Parsons et al., 1997; Johnson et al., 1998; Wang and Parsons, 1998). Nengas et al. (1999) found that locally available PBM caused severe growth depression in red sea bream whereas a superior grade of PBM caused only slight reductions in growth. Bureau et al. (1999) observed high variation in both quality and digestibility of a variety of poultry products in rainbow trout due to processing method. Dong et al. (1993) noted significant differences in chemical composition as well as protein digestibility of PBM in salmonids depending on the product source. Another limitation proceeds from factors associated with diet formulation. Attention to the levels and final ratios of IAA in the diet is often lacking after fishmeal has been totally or partially replaced and are underlying reasons that PBM-based diets fail to perform like fishmeal-based control diets (Abdel-Warith et al., 2001; Millamena and Golez, 2001; Emre et al., 2003; Turker et al., 2005).

Two possible strategies to circumvent these limitations are use of higher quality by-products and modification of the upper bounds of least-cost formulas with regard to amino acid supplementation. Due to added controls during offal collection and processing, petfood-grade PBM is a high quality, low-ash by-product of more uniform composition than its FAQ (fair and average quality) counterpart (Miller, 1996). Bureau et al. (1999) noted significant improvements in the digestibility of nutrients in PBM as compared to earlier work (Cho and Slinger, 1979). Secondly, results of comparative fish-meal replacement studies in trout and turbot suggest that formulating to the putative amino acid requirements of a species of interest is insufficient (Fournier et al., 2003, 2004). Livestock diets are often formulated on an ideal protein basis, i.e. formulated to meet an "ideal" amino acid profile based on the pattern found in a generally accepted tissue model (Fuller et al., 1979). Mambrini and Kaushik (1995) suggested that the amino acid profile of fish meal might be a suitable model for fish and that formulating on this basis would necessitate supplementation of IAA above published requirement estimates. Indeed, Twibell et al. (2003) demonstrated that increasing the dietary IAA content to match the amino acid profile of herring meal improved the growth of hybrid striped bass above that observed for fish fed a diet formulated to meet the IAA requirements.

We recently demonstrated that petfood-grade PBM effectively replaced 100% of fishmeal in semi-purified diets for hybrid striped bass when substituted on an ideal protein basis (Gaylord and Rawles, 2005). In that study, Met and Lys supplementation of a diet in which all protein was provided by PBM ameliorated deficiencies in hybrid striped bass production when fish were fed the unsupplemented PBM diet. Moreover, fish fed the supplemented PBM diet exhibited equivalent weight gain and feed efficiency as fish fed a diet in which all protein was supplied by fishmeal. The purpose of this study was to address the efficacy of commercial fishmeal replacement diets for hybrid striped bass that are formulated using a similar strategy.

2. Materials and methods

2.1. Commercial test diets

A generic commercial production diet (GEN) for hybrid striped bass was formulated to contain 45% protein, 12% lipid and 3.7 kcal/kg estimated available energy (Table 1). Protein in the GEN diet was supplied by a mix of animal and plant sources (Burris Aquaculture and Specialty Feeds, Franklinton, LA) that included more than 20% select menhaden fishmeal (MFM) and less than 10% PBM, and are considered typical of commercial formulations. Digestible protein and available Met and Lys in the GEN diet from MFM and PBM were estimated from the data of Gaylord and Rawles (2005). Since the availability of IAA to *Morone* spp. had not been determined for the remaining dietary protein sources, gross Met and Lys levels expected in the remaining ingredients (NRC, 1993) were added to available Met and Lys from MFM and PBM in order to approximate total levels of these amino acids in the GEN formula.

Table 1
Composition (% as-fed) of commercial test diets fed to hybrid striped bass in recirculated tanks for 24 weeks

Ingredient	Diet[a]			
	GEN	GEN+AA	35PBM	70PBM
Menhaden fishmeal	25.00	25.00	16.20	7.50
Poultry by-product, petfood grade	7.73	7.73	17.20	26.68
Soybean meal 48%	25.90	25.90	25.90	25.90
Wheat midds	17.35	11.72	9.73	7.43
Wheat	8.00	11.14	12.43	13.92
Cottonseed meal	4.80	4.80	4.80	4.80
Rice bran	3.80	3.80	3.80	3.80
Menhaden fish oil	6.94	7.09	6.54	5.99
Vitamin premix	0.35	0.35	0.35	0.35
Trace mineral premix	0.07	0.07	0.07	0.07
Stay-C 35%	0.05	0.05	0.05	0.05
Ethoxyquin dry	0.01	0.01	0.01	0.01
Lysine–HCl		1.42	1.55	1.67
DL-Methionine		0.58	0.63	0.68
Analyzed composition [b]				
Moisture (%)	8.3	9.0	8.6	9.3
Protein (%)	41.2	42.7	43.2	43.0
Lipid (%)	11.1	11.5	10.8	9.6
Ash (%)	10.8	10.6	10.4	9.6
Gross energy (kcal/kg)	4598	4569	4647	4673
Digestible energy (kcal/kg)	3211	3292	3164	3429

[a] Diet designations are as follows: GEN=generic commercial diet, GEN+AA=generic diet with supplemental Met and Lys (+AA), 35PBM=35% replacement of fishmeal with poultry by-product+AA, 70PBM=70% replacement of fishmeal with poultry by-product+AA. (Ingredients provided by Burris Feeds, Inc., Franklinton, Louisiana).
[b] As-fed basis.

A positive control diet (GEN+AA) was formulated by supplementing the GEN diet with feed-grade Met and Lys to match levels in hybrid striped bass muscle at 40% digestible protein (Table 1). Substitution diets were formulated by replacing 35% or 70% of MFM in the GEN diet with PBM (designated 35PBM and 70PBM, respectively) on a digestible protein basis and then supplementing with Met and Lys as needed to match those levels in the positive control.

Diet formulation and extrusion were performed by a commercial mill (Burris Aquaculture and Specialty Feeds, Franklinton, LA) and all diets met or exceeded known nutritional requirements for hybrid striped bass. Upon exiting the dryer, feed pellets were sealed in standard commercial wax-lined bags (22.5 kg/bag) and shipped to the Aquaculture Division of Harbor Branch Oceanographic Institution, Ft. Pierce, FL where they were stored in a temperature-controlled building until fed.

2.2. Recirculated culture system and fish

The feeding trial was conducted in three replicate recirculated culture systems. Each system consisted of four 8-m^3 dual drain round culture tanks with a total volume of 45 m^3 per system. Bottom drains were plumbed directly to swirl separators. Swirl separators and tank side drains emptied into a common manifold effluent line. The effluent line emptied into a 60-μm drum filter (Hydrotech HDF800-1P, Velllinge, Sweden) and then into a sump. Each sump was connected to a manifold consisting of three 2-hp pumps (Whisperflo WFK-8, Pentair Pool Products, Sanford, NC). Water from the sump was pumped through two 0.71-m^3 propeller wash bead filters (PBF-25, Aquaculture Systems Technologies, New Orleans, LA) to a T-junction. At the T-junction, filtered water could be directed to the tanks or returned to the sump. Water continuing to the tanks passed through an oxygen cone and ultraviolet light disinfection system (COM8 520 watt, Emperor Aquatics, Pottstown, PA). Water returning to the sump passed through a stacked tray de-gas column. The three systems were housed in a 418-m^2 insulated building. Building temperature was regulated using thermostat-controlled central heating and cooling. Propane heaters were used to increase temperature and exhaust fans were used to rapidly decrease temperature.

Reciprocal cross hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) fry (2 g/fish average initial weight) were obtained from a commercial producer (Keo Fish Farms, Keo, AR) and stocked in a flow-through quarantine system for 47 days. Juvenile fish were transferred to a recirculating nursery system for 103 days. During the 150-day period, fish were fed a commercial trout diet. At the conclusion of the acclimation period, 300 fish (87 g/fish mean initial weight) were randomly stocked into each of the 12 tanks comprising the three replicated culture systems. One tank of fish in each of the three systems was randomly assigned one of the four diets in a randomized block design. Diets were initially fed at 4% body weight day^{-1} divided into two equal (morning and afternoon) feedings for the first 8 weeks of the study and then gradually decreased by 0.5% body weight day^{-1} every 4 weeks thereafter to a final feeding rate of 1.5% body weight day^{-1}. The feeding trial lasted 24 weeks. Random samples of 30 fish/tank were netted, weighed and returned to the tank at 28-day intervals to adjust tank rations.

Water quality was evaluated daily for oxygen, pH, salinity, temperature, ammonia-nitrogen (TAN), nitrite-nitrogen (NO$_2$-N) and total alkalinity, and weekly for nitrate-nitrogen (NO$_3$-N) and carbon dioxide. Measurements were taken at 14:00 h. Water quality

characteristics were maintained within acceptable levels for hybrid striped bass culture (Warren et al., 1990). Specifically, oxygen ranged from 6 to 15 mg/l, pH from 6.8 to 8.2, temperature from 22 to 28 C, salinity from 0.5 to 1.5 g/l, TAN from 0 to 2 mg/l, NO_2-N from 0 to 5 mg/l, total alkalinity from 150 to 200 mg/l, NO_3-N from 0 to 100 mg/l and carbon dioxide from 0 to 50 mg/l. Water flow was regulated to provide 1 to 1.5 tank turnovers/h. Exchange of new water was less than 3% of the system volume per day. System salinity reflected that of the incoming water and total alkalinity was adjusted daily by the addition of sodium bicarbonate.

2.3. Digestibility trial

The digestibility of gross nutrients, energy and IAA in the commercial test diets were determined in a separate trial at the USDA/ARS Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR. The digestibility diets contained 99% commercial test diet +1% marker (Cr_2O_3) on a dry weight basis. Aliquots of the commercial test diets were ground, mixed with marker and pressure-pelleted according to methods previously described (Gaylord and Rawles, 2005). Pellets were dried to less than 10% moisture in a forced-air conveyor oven (Pacesetter PS250, Middleby-Marshall, Morton Grove, IL) at 250 C for 17 min and stored at −20 C until fed. Four replicate tanks of fish were randomly assigned to each diet. Tanks were stocked with 50 hybrid striped bass each that averaged 125 g/fish. Fecal collection and calculation of digestibility coefficients were conducted according to Gaylord et al. (2004).

2.4. Compositional indices and nutrient retention

At the termination of the growth trial, all fish were counted and weighed. Ten fish per tank were euthanized and dissected to determine body condition indices. Condition indices were calculated according to the following:

Muscle ratio (MR)=fillet mass with ribs * 100 / fish mass
Hepatosomatic index (HSI)=liver mass * 100 / fish mass
Intraperitoneal fat ratio (IPF)=peritoneal fat mass * 100 / fish mass

Ten randomly selected fish were collected and frozen at the beginning of the trial and an additional five fish per tank were frozen at the end of the trial for later analysis of whole body protein and energy. Frozen fish were sectioned and passed through an industrial meat grinder. Ground sections were pooled and thoroughly mixed prior to each regrinding for an additional two times. Whole body protein and energy retention efficiencies were calculated according to the following formulas:

Protein retention efficiency (PRE)=protein gain * 100 / protein fed
Energy retention efficiency (ERE)=energy gain * 100 / energy fed

Retention efficiencies were calculated on a dry-weight basis. Animal care and experimental protocols were approved by the Harry K. Dupree Stuttgart National Aquaculture Research Center Institutional Animal Care and Use Committee and conformed to Agricultural Research Service Policies and Procedures 130.4 and 635.1.

2.5. Chemical analyses

Proximate (dry matter, ash, protein and lipid), chromium, energy and amino acid contents of ingredients, diets and relevant tissues were performed according to standard methods (AOAC, 2000). Briefly, protein ($N \times 6.25$) was determined by the Dumas method using a LECO nitrogen analyzer (FP428, LECO Corporation, St. Joseph, MI). Lipid was determined according to Folch et al. (1957) following chloroform–methanol (2:1) extraction. Amino acids in diets and fecal samples were determined by a commercial laboratory (Central Analytical Laboratory, University of Arkansas, Fayetteville, AR) using high performance liquid chromatography and AOAC (2000) Official Method 982.30, Part E. Briefly, amino acids except tryptophan and sulfur amino acids were determined after acid hydrolysis. Sulfur amino acids were determined separately after performic acid oxidation and acid hydrolysis. Tryptophan was determined separately after alkaline hydrolysis. Chromium was determined after perchloric acid oxidation of ashed samples using the diphenylcarbazide (DPC) colorimetric method as described in Divakaran et al. (2002). Total energy was determined by isoperibol bomb calorimetry (Parr1281, Parr Instrument Company, Moline, IL).

2.6. Statistical analyses

The SAS software program PROC MIXED (SAS Institute, 2002) was used to conduct a mixed effects model analysis of variance (Ott, 1977) of observed apparent digestibility coefficients (ADC) for dry matter, organic matter, protein, lipid, gross energy and digestible energy (DE), and apparent availability coefficients for

Table 2
Apparent digestibility (%) of nutrients and energy and amino acid availability (%) in commercial test diets for hybrid striped bass

| | ADC | | | | | |
	GEN[a]	GEN+AA	35PBM	70PBM	Pooled S.E.	P>F
Organic matter	65.4	67.5	62.4+	69.1	1.49	0.029
Protein	97.2	97.2	96.7	97.2	0.21	0.091
Lipid	85.3	87.2	87.0	89.9	0.83	0.058
Energy	70.4	72.4	69.7	73.6	1.33	0.168
Amino acids						
Arg	96.2	94.3	93.6	95.0	0.85	0.150
Cys	48.1	49.0	41.6	48.0	4.93	0.377
His	88.3	86.7	83.5	86.5	1.55	0.052
Ile	88.7	87.6	81.8*+	87.1	1.49	0.030
Leu	90.8	89.0	88.9	89.3	1.22	0.178
Lys	90.8	91.7	91.0	92.5	0.84	0.727
Met	88.1	90.1	88.4	90.2	0.98	0.444
Phe	89.9	89.4	86.6*+	88.9	0.84	0.003
Thr	86.2	84.1	74.4*+	81.3*	1.91	<0.001
Trp	93.4	88.5	87.7	86.3	2.80	0.125
Tyr	89.9	88.4	83.9*+	87.7*	1.06	<0.001
Val	87.5	86.0	83.8*	85.3	1.13	0.013

Values are mean apparent digestibility coefficients (ADC, dry-weight basis) from four replicate tanks of fish. An asterisk indicates the mean is different ($P<0.05$) from that of the generic diet (GEN). A plus sign indicates the mean is different ($P<0.05$) from that of the amino acid supplemented generic diet (GEN+AA). Differences between treatment means were separated using contrast statements and Bonferroni *t*-tests (Miller, 1981) for pair wise comparisons to control type I error.

[a] Diet designations are described in Table 1.

amino acids in the commercial test diets (fixed effects). Tank within test diet was defined as the random effect with compound symmetric variance–covariance structure. Contrast statements were constructed to compare the digestibility of nutrients and energy in the substitution diets (35PBM and 70PBM) to the digestibility of nutrients and energy in the generic (GEN and GEN+AA) diets. Similarly, a mixed effects ANOVA was conducted on the growth trial response data in which diet was defined as the fixed effect and culture system was defined as a random effect with compound symmetric variance–covariance structure. The interaction of diet with culture system was not testable since each test diet was fed to only one tank of fish in each culture system. Contrast statements were employed to compare responses to amino acid supplemented replacement diets with responses to the supplemented and unsupplemented generic diets. In all statistical analyses, differences among treatment means were separated using Bonferroni *t*-tests (Miller, 1981) for pair-wise comparisons in order to control type I error. Treatment effects were considered significant at $P<0.05$.

3. Results

3.1. Digestibility trial

Apparent digestibility coefficients of gross nutrients and energy were generally moderate to high and fairly uniform among the commercial test diets (Table 2). Gross nutrients and energy in the 35PBM diet appeared slightly less digestible than nutrients and energy in the generic diets, while the opposite tendency seemed evident for nutrients and energy in the 70PBM diet; however, these trends were significant only for organic matter and marginally significant ($P=0.058$) for lipid. The digestibility of organic matter was moderately high and ranged from 62% in the 35PBM diet to 69% in the 70PBM diet. The digestibility of protein was both high and uniform (mean±S.D., 97.0±0.5%) among all test diets, as was the digestibility of energy (71.0±2.9%). Digestible energy (DE) was not significantly different ($P=0.086$) among diets and varied from 3164 kcal/kg in the GEN diet to 3429 kcal/kg in the 70% PBM diet (Table 1).

Table 3
Comparison of the indispensable amino acid profiles (dry-weight basis) of an ideal protein model and commercial fishmeal replacement diets for hybrid striped bass containing petfood grade poultry by-product and supplemental Met and Lys

| | Ideal protein[a] | Available dietary IAA[b] | | | |
		GEN	GEN+AA	35PBM	70PBM
Amino acids	*(g/100 g diet)*				
Arg	4.12	3.16	2.90	3.03	3.06
His	1.31	1.00	0.97	0.93	0.97
Ile	1.87	1.83	1.77	1.63	1.80
Leu	3.02	3.10	2.98	3.00	3.11
Lys	3.47	2.70	3.92	3.92	4.38
Met	1.32	1.17	1.98	1.96	2.06
Phe	1.68	1.86	1.83	1.73	1.88
Thr	2.33	1.41	1.37	0.96	1.21
Trp	0.40	0.33	0.30	0.33	0.30
Val	2.10	2.14	2.05	2.04	2.13
TSAA[c]	1.84	1.52	2.33	2.28	2.43
TAAA[d]	3.20	2.95	2.91	2.64	2.91
Selected amino acid ratios					
Arg/Lys	1.19	1.17	0.74	0.77	0.70
Ile/Leu	0.62	0.59	0.59	0.54	0.58
Val/Leu	0.70	0.69	0.69	0.68	0.69
TSAA/Lys	0.53	0.56	0.59	0.58	0.56

[a] Values are based on a hypothetical diet containing 40% digestible protein from hybrid striped bass muscle (Gaylord and Rawles, 2005).
[b] Values are based on analysis and availability coefficients obtained in the digestibility trial (Table 2). Diet designations are described in Table 1.
[c] Total sulfur amino acids.
[d] Total aromatic amino acids.

Table 4
Response of hybrid striped bass fed commercial fishmeal replacement diets containing petfood grade poultry by-product and supplemental Met and Lys for 24 weeks

	Diet[a]				Pooled S.E.	$P>F$
	GEN	GEN+AA	35PBM	70PBM		
Initial weight (g/fish)	87.2	87.0	87.6	87.0	0.69	0.904
Final weight (g/fish)	787[+]	825*	775[+]	705*[+]	10.58	0.001
Weight gain (%)[a]	800	841	779[+]	709*[+]	15.18	0.003
Yield (kg)	235	246	231[+]	211*[+]	3.26	0.002
FCR[b]	1.66	1.58	1.65	1.72	0.03	0.093
Survival (%)	98.7	98.3	98.9	99.3*[+]	0.40	0.043
Whole body protein (% dry wt)	53.9	54.4	49.6*+	49.0*+	0.95	0.013
PRE (%)[c]	29.0	30.1	28.8	27.0	0.74	0.072
Whole body energy (kcal/g)	6.219	6.266	6.483	6.401	0.071	0.181
ERE (%)[d]	30.4	32.5	34.6	33.1	1.19	0.259
HSI (%)[e]	2.05	2.08	2.06	2.43*+	0.19	<0.001
IPF ratio (%)[f]	6.81	6.44	7.64*+	8.50*+	0.28	<0.001
Muscle ratio (%)[g]	35.7	35.8	36.0	35.9	0.60	0.986

An asterisk indicates the mean ($N=3$) is different ($P<0.05$) from that of the generic diet (GEN). A plus sign indicates the mean is different ($P<0.05$) from that of the amino acid supplemented generic diet (GEN+AA). Differences between treatment means were separated using contrast statements and Bonferroni t-tests (Miller, 1981) for pair-wise comparisons to control type I error. Diet designations are described in Table 1.

[a] Weight gain=(Final weight−initial weight)*100/initial weight.

[b] Food conversion ratio (FCR)=g feed fed/g weight gained.

[c] Protein retention efficiency (PRE)=protein gain*100/protein fed; values are mean determinations (dry-weight basis) on five fish per tank ($N=3$ tanks/treatment).

[d] Energy retention efficiency (ERE)=energy gain*100/energy fed; values are mean determinations (dry-weight basis) on five fish per tank ($N=3$ tanks/treatment).

[e] Hepatosomatic index (HSI)=liver mass×100/fish mass; values are mean determinations (fresh-weight basis) on ten fish per tank ($N=3$ tanks/treatment).

[f] Intraperitoneal fat (IPF) ratio=peritoneal fat mass*100/fish mass; values are mean determinations (fresh-weight basis) on ten fish per tank ($N=3$ tanks/treatment).

[g] Muscle ratio=fillet mass*100/fish mass; values are mean determinations (fresh-weight basis) on 10 fish per tank ($N=3$ tanks/treatment).

The availabilities of amino acids in the test diets generally exceeded 80% (Table 2) and were fairly uniform (maximum S.D.=5.6%). The notable exception was Cys availability that was half that of the other amino acids in the test diets and highly variable (46.7±9.9%). Of the 10 IAA, Ile, Phe, Thr and Val availability differed among diets. Generally, differences were due to lower availabilities of these amino acids in the 35PBM diet. Specifically, the availability of Val in the 35PBM diet was less than that of GEN, while the availabilities of Ile, Phe, Thr and Tyr in the 35PBM diet were less than those of both GEN and GEN+AA. The availabilities of both Thr and Tyr in the 70PBM diet were less than those of GEN as well.

All diets in the current study were replete with respect to published amino acid requirements of hybrids striped bass. Compared to an ideal protein model of 40% protein provided by hybrid striped bass muscle, however, the available IAA composition of the GEN diet was first-limiting in Arg followed by Thr, Lys and His (Table 3). All diets supplemented with Lys and Met (GEN+AA, 35PBM, 70PBM) were replete for those amino acids with respect to the ideal protein model. Therefore, His became third-limiting in the supplemented diets, while Thr

or Arg were first- or second-limiting, depending on whether or not PBM was substituted for fishmeal. The ratios of selected amino acids also changed among the commercial test diets. The ratio of available Arg to available Lys in the GEN diet (1.17) was similar to that of the ideal protein model (1.19), whereas the ratios of Arg/Lys in the supplemented diets were half of those in the generic diet or ideal model. Ratios of branched chain amino acids did not differ significantly among the test diets. The ratio of available Ile to available Leu ranged from a high of 0.62 in the ideal model to a low of 0.54 in the 70PBM diet, whereas the ratio Val/Leu only slightly declined from a high of 0.70 in the ideal model to a low of 0.68 in the 70PBM diet. The ratio of available total sulfur amino acids (TSAA) to available Lys, however, was lowest in the ideal protein model (0.53) and highest in the 70PBM diet (0.58).

3.2. Growth performance

Initial weight of fish stocked in the growth trial did not differ among dietary treatments and averaged 87.2± 1.0 g (Table 4). Weight gain and final fish weight were significantly affected by dietary treatment (Table 4).

Final weight (775 g) and weight gain (779%) of fish fed the 35PBM did not differ from that of fish fed the GEN diet (787 g and 800%, respectively), but were less than that of fish fed the GEN + AA diet (825 g and 841%, respectively). Fish fed the 70PBM weighed less (705 g) and gained less weight (709%) than fish fed either generic diet. Fish fed the GEN diet weighed less at the end of the trial than fish fed the GEN + AA diet; however, weight gain was not different between generic diets.

Final yield of fish fed the 35PBM diet (231 kg) was not different from final yield of fish fed the GEN diet (235 kg), but was less than that (246 kg) of fish fed the GEN + AA diet (Table 4). Final yield of fish fed the 70PBM diet (211 kg) was significantly less than yields from either generic diet treatment. Food conversion ratios (FCR) from fish fed the PBM replacement diets did not differ from fish fed the generic diets (Table 4). Survival was higher in the 70PBM dietary treatment; however, survival among all treatments was greater than 98% with a range of only 1 point (Table 4).

3.3. Compositional indices and nutrient retention

Whole body protein content of fish fed either the 35PBM (49.6%) or 70PBM (49%) diets was less than whole body protein content of fish fed either the GEN (53.9%) or GEN + AA (54.4%) diets, whereas PRE did not differ among dietary treatments (Table 4). Whole body energy content and retention efficiencies of fish fed the PBM replacement diets did not differ from that of fish fed the generic diets (Table 4).

Body indices of energy deposition were affected by replacement of fishmeal with PBM; however, muscle ratio (35.7–36.0%) did not differ among dietary treatments (Table 4). HSI of fish fed the 70PBM diet (2.43%) was greater than HSI of fish fed the GEN diet (2.05%) or the GEN + AA diet (2.08%), whereas HSI of fish fed the 35PBM (2.06%) did not differ from that of fish fed either generic diet. IPF ratios in fish fed the PBM replacement diets were higher than IPF ratios in fish fed either generic diet.

4. Discussion

The reduced performance of hybrid striped bass fed the 70PBM diet suggests a limit between 35% and 70% substitution of fishmeal protein with that of petfood-grade poultry by-product under the assumptions and conditions of the present study. Since the generic diets already contained a base level of PBM that contributed 5% crude protein toward total protein in the diet, this amounts to between 11% and 18%, or nearly half, of the

dietary protein that can be provided by PBM and supplemental Met and Lys. Abdel-Warith et al. (2001) found that up to 40% of protein in practical diets for African catfish (or 15% total crude protein) could be provided by PBM before significant reductions in performance were observed. Replacing all protein provided by fishmeal (24.5% total crude protein) with PBM resulted in only slight reductions in performance indices of tilapia and a large increase in the profit index for the replacement diets (El-Sayed, 1998). Hybrid tilapia fed a replacement diet in which PBM provided 17% crude protein, or 60% of dietary protein, exhibited equivalent weight gains, feed conversions and protein efficiencies as fish fed a fishmeal-based control diet (Fasakin et al., 2005). Juvenile red drum were able to use up to 13% crude protein or 30% of the total dietary protein provided by either PBM or enzyme-digested PBM with no differences in performance with respect to fish fed a fishmeal-based control diet (Kureshy et al., 2000). Nengas et al. (1999) were able to replace 50% of the protein provided by fishmeal, or 18% crude protein (40% of dietary protein), with PBM in diets for gilthead seabream; however, reductions in growth performance were seen at the 75% level of fishmeal replacement. Similarly, Quartararo et al. (1998) were able to replace 27% of the protein provided by fishmeal, or 12% crude protein, with protein from PBM and supplemental Met (0.34%) and Lys (0.24%) in diets for Australian snapper, but reductions in weight gain and specific growth rate were evident at higher levels of replacement. Nevertheless, an economic analysis revealed that up to 50% replacement of fishmeal protein with PBM was cost-effective in Australian snapper diets in spite of the reduced performance at that level of substitution. Yang et al. (2004) also found PBM was able to replace 50% of the protein provided by fishmeal (19% crude protein) in diets for gibel carp. Bharadwaj et al. (2002) replaced 24% crude protein from fishmeal with Met (0.25% of diet) and a meat and bone mixture that contained nearly 40% PBM and found only slight differences in performance measures of hybrid striped bass, although overall feed consumption (2.3% of body weight) and growth (109% to 140% in 7 weeks for 55 g fish) in the trial seems low. On the other hand, Emre et al. (2003) observed drastic reductions in growth and nutrient retention of mirror carp fingerlings fed diets containing as little as 33% PBM without amino acid supplementation.

The positive correlation of major depots of dietary energy (HSI and IPF) with level of PBM in the diet observed in the current study is also evident in previous work. El-Sayed (1994, 1998) noted higher carcass lipid in silver seabream and Nile tilapia, respectively, when PBM

was substituted for fishmeal than when other protein sources were substituted. Steffens (1994) found increasing body lipid and HSI, and Zoccarato et al. (1996) observed increasing HSI and viscerosomatic index in rainbow trout as level of PBM in the diet increased.

The reduced performance of fish fed the 70PBM diet is disappointing in light of our previous work (Gaylord and Rawles, 2005). In that study, Met and Lys were found to be first- and second-limiting in PBM and deficiencies in hybrid striped bass performance were ameliorated by supplementing a 100% replacement diet with sufficient Met and Lys to match levels in a hypothetical diet in which all protein (40%) was provided by hybrid striped bass muscle. As in our previous work, levels of Met and Lys in the supplemented diets of this study exceeded the published requirements. Several explanations are commonly advanced when fishmeal replacement diets fail to perform as expected including a nutrient deficiency, lower digestibility, or an imbalance of energy or nutrients in the substituted ingredient, and antinutritional factors in the substitute that may have reduced intake or interfered with metabolism.

Digestibility coefficients of nutrients were moderate to high as well as uniform among all diets in the current study. In particular, protein digestibility was greater than 90%. High protein digestibility is often correlated with high indispensable amino acid availability (Wilson, 2002). With the exception of Cys, amino acid availabilities in this study exceeded 80% and were consistent among the test diets for any particular amino acid. The availability of Cys, however, was half that of the other amino acids in the test diets. A number of workers have observed Cys availabilities that are roughly half that of other amino acids in various ingredients, including meat meal in yellowtail diets (Masumoto et al., 1996), fish and poultry by-product meals in salmon diets (Portz and Cyrino, 2004), and brewer's by-products in trout diets (Cheng et al., 2003). High temperatures during ingredient processing or diet manufacture are commonly cited explanations for the reduced availability of amino acids in various ingredients. In contrast to our previous study (Gaylord and Rawles, 2005), the diets in the current study were commercially extruded and it is possible that the heat of extrusion affected Cys availability. Sørensen et al. (2002) noted that Arg, Cys, Lys, Ser and Thr are most likely to be damaged by processing temperature. However, with the exception of Cys, those authors were unable to detect differences in amino acid availability to rainbow trout due to changes in extrusion temperature.

Differences in the PBM obtained for this study, as opposed to our previous study, may have been a factor in the poorer performance of the 70PBM diet. An essential condition of the current study was that diet formulation and manufacture be carried out in a relevant commercial setting in which different batches of a particular ingredient are received and combined prior to diet mixing and extrusion. In the commercial context, the availability of specific amino acids within an ingredient can vary considerably among different batches of that ingredient. In examining the availability of amino acids in different samples of high-quality meat and bone meal, for example, Parsons et al. (1997) found that variability was greatest for Cys (37–72%) and Lys (69–88%) and least for Thr (72–86%) and Met (84–89%) than for all other amino acids examined. Also, high nutrient digestibility among test diets is not always a good indicator of expected diet performance. Kissil et al. (2000) found no differences in protein availability among fishmeal replacement diets for gilthead seabream in spite of reduced performance at higher inclusion levels of soy and rapeseed protein concentrates.

Nevertheless, the tendencies for whole body protein to decrease and energy depots (HSI and IPF) to increase as PBM replaced fishmeal indicates a nutrient imbalance in the replacement diets. In contrast to our previous work, the current study employed diets containing a typical mix of animal and plant protein sources. Because amino acid availabilities in a particular product tend to be species specific (compare, for example, the data of Portz and Cyrino, 2004 with that of Gaylord and Rawles, 2005), we did not apply availability coefficients from other species for products for which no availability data existed in HSB. The commercial test diets, therefore, are hybrid formulations based on available amino acids with respect to PBM and fishmeal and estimated total amino acid composition with respect to the plant products. As the diet composition and availability data reveal, the available concentrations of some IAA in the replacement diets were not optimum when compared to the ideal model. It is also noteworthy that digestible energy (DE) was numerically higher in the 70PBM diet, since requirements for IAA increase as DE increases (Wilson, 2002). Hence, the marginally higher ($P=0.08$) DE of the 70PBM diet may have exacerbated imbalances in available indispensable amino acids and contributed to the reduced performance of this diet.

The level of available Met in the GEN diet was lower than that of the ideal protein, but was not, contrary to expectation, first-limiting when compared to the IAA profile of the ideal protein model. Instead, Arg appeared to be first-limiting, Thr was second-limiting, Lys was third-limiting, and His was fourth-limiting. These data are in sharp contrast to our previous study in which Met and Lys were found to be first- and second-limiting when all protein in diets for HSB was provided by PBM (Gaylord and Rawles, 2005). In the supplemented

replacement diets, levels of available Met and Lys exceeded those of the ideal model, Thr appeared to be first-limiting, Arg appeared to be second-limiting, and His was third-limiting. In replacement diets for gilthead seabream, Nengas et al. (1999) suggested that Met was first-limiting in a high-fat PBM, whereas His was first-limiting in a high-protein PBM, based on total amino acid content. However, the identification of limiting amino acids according to total or available amino acid content may not agree with results from growth trials. Based on the ideal amino acid pattern for finishing pigs, TSAA in sorghum-soybean-based diets was first- or second-limiting, whereas Lys and Thr were first- and second-limiting in growth trials (Knowles et al., 1998).

The fact that much lower content of Met and Lys were predicted in the test diets by the commercial formulas underscores the value of formulating on an available, rather than total, amino acid basis and numerous studies concur (e.g., Fernandez et al., 1995; Rostagno et al., 1995). In the current study, formulation on an available amino acid basis would have resulted in virtually no Met, and lower levels of Lys, supplementation. Wang and Parsons (1998) found that adding one more ingredient to a corn-sorghum-based diet on a total amino acid basis depressed performance in chicks, whereas adding the same ingredient on an available amino acid basis resulted in little or no negative effect. In addition, if the GEN diet represents a typical production formula for HSB, then the superior performance of the supplemented generic diet (GEN+AA) suggests that significant improvements can be made in current hybrid striped bass feeds by formulating on an ideal protein basis. The profile of available IAA in the GEN diet, for example, indicated that Lys was third-limiting with respect to the ideal protein model. Hence, it is reasonable that adding Lys and increasing the digestible protein content caused the overall improved performance of fish fed the GEN+AA diet. In a related scenario, supplementing fishmeal-based diets of low digestible protein (DP) to DE ratio with IAA at 1.2 times the requirement resulted in feed efficiencies, whole body protein content, nitrogen retention and fat content of rainbow trout that were equivalent to fish fed a similar diet of optimum DP/DE ratio (Yamamoto et al., 2005), whereas the same performance measures were poorer in trout fed the low DP/DE ratio diets without IAA supplementation.

One obvious concern in the current study is whether Lys supplementation of the test diets contributed to lower Arg utilization and poorer performance of fish fed the 70PBM diet. Evidence of dietary Lys–Arg antagonism is well documented in livestock production (D'Mello, 1994) but varies considerably among species of fish (Wilson, 2002) and the level of metabolism at which the Arg–Lys

interaction is studied (Berge et al., 2002). For example, radiotracer data indicated Lys inhibition of Arg availability in Atlantic salmon muscle; however, growth and feed utilization actually increased in fish fed diets marginal in Arg and supplemented with high levels of Lys (Berge et al., 2002). The data of Tibaldi et al. (1994) and Griffin et al. (1994) suggested that European sea bass, *Dicentrarchus labrax*, as well as hybrid striped bass, respectively, were relatively insensitive to Arg/Lys ratio in the diet. In the current study, Arg appeared to be second-limiting in the replacement diets and the ratios of available Arg/Lys were much less than that of the ideal protein and declined as the level of PBM substitution increased. In particular, the difference between available Lys in the 70PBM diet and Lys in the ideal protein was double the difference between Lys in the 35PBM diet and Lys in the ideal protein (0.91 g/100 g diet vs. 0.45 g/100 g diet, respectively), i.e., there was twice as much available Lys in the 70PBM diet than in the 35PBM diet when compared to the ideal protein. At the same time, the ratio of available Arg/Lys decreased from 0.77 in the 35PBM diet to 0.70 in the 70PBM diet, while growth performance and compositional indices of fish fed the 70PBM diet were much poorer than those of the 35PBM diet. Nevertheless, Lys supplementation in the GEN+AA diet resulted in superior final weight and similar compositional indices to fish fed the GEN diet even though the Arg/Lys ratio of the GEN+AA diet (0.74) was similar to those of the other supplemented diets and lower than that of the ideal model (1.19) or GEN diet (1.17). Taken together, these data also suggest that HSB are relatively insensitive to Arg–Lys antagonism, although this is not clearly demonstrated in the current experiment. In the future, the potential antagonisms between Arg and Lys may require further consideration in HSB when formulating diets on an ideal protein basis and supplementing crystalline Lys.

The hypothesis that Thr was first-limiting in the commercial replacement diets is consistent with our previous findings. Gaylord and Rawles (2005) suggested Thr would become first-limiting and Leu second-limiting after the ideal requirements for Met and Lys were met. In fact, the addition of Thr without Leu actually reduced performance in hybrid striped bass in that study and implied that the balance of branched-chain amino acids (BCAA) might become critical for hybrid striped bass fed diets containing a mix of protein sources. Both Robinson et al. (1984) and Yamamoto et al. (2004) noted it was critical to balance dietary BCAA for fingerling catfish and rainbow trout, respectively. In the latter study, imbalances accrued as increasing proportions of plant proteins replaced fishmeal protein. However, available levels of Ile, Leu and Val in this study were within +170% of

published requirements and within +20% of concentrations in the ideal model. Robinson et al. (1984) found no reduction in channel catfish growth and feed efficiency when dietary levels of BCAA were 200% of their requirement. Additionally, the ratios of Ile/Leu and Val/Leu in the test diets were within 1% and 2%, respectively, of the ideal model, whereas reduced performance of trout was elicited only when imbalances in dietary BCAA were 50% of an ideal model (Yamamoto et al., 2004). Therefore, it is unlikely that imbalances in BCAA were significant factors in the poorer performance of the 70% replacement diet.

Another concern would be a potential imbalance in the total sulfur amino acid (TSAA) content and/or the TSAA/Lys ratio in the replacement diets. Available TSAA levels in the replacement diets were greater than that of the ideal model and the greatest difference (+132%) was observed in the 70PBM diet. At the same time, the ratio of available TSAA/Lys in the 70PBM diet exhibited the smallest percent change (+4.5%) from that of the ideal model due to the increased availability of Lys in the 70PBM diet. To date, little work has been done to address the optimum TSAA/Lys ratio in practical diets for fish.

Fish are able to compensate to a certain degree for imbalances in dietary amino acid composition by modifying intake. Yamamoto et al. (2000) demonstrated that rainbow trout discriminated between self-feeders containing diets of unbalanced, versus balanced, amino acid composition by preferentially choosing feeders containing the balanced diet. In a subsequent study, trout were shown to repeatedly increase intake of a balanced diet and decrease intake of the same diet deficient in only Lys and yet were unable to demonstrate the same capacity with respect to a diet deficient in only Met (Yamamoto et al., 2001). The commercial test diets of the present study, however, were offered at a constant, but slowly declining, percentage of body weight in order to approximate a level of intake slightly below satiation—4% of body weight initially, which was gradually reduced to 1.5% over the course of the 24-week trial. Caretakers noted an increase in feeding time in tanks of fish receiving the replacement diets during the last 3 to 4 weeks of the trial; however, the potential difference between actual feed intake with respect to feed offered on the basis of body weight was not measured in the current study. Nevertheless, these observations suggest a potential problem in the palatability of the diets with higher amino acid supplementation.

Although palatability of high-quality poultry by-products has not been a prevalent issue in replacement studies with carnivorous fish, dietary supplements can reduce intake. In the current study, supplementation of Met and Lys resulted in dietary levels that exceeded those of the ideal model and provided more than 400% and 200%,

respectively, of the published requirements for HSB. Fish vary in their response to supplemental amino acids, while trout (Rodehuteschord et al., 1995; Cheng et al., 2003), catfish (Bai and Gatlin, 1994; Reigh, 1999) and seabream (Gómez-Requeni et al., 2004) respond well to amino acid supplementation, carp apparently do not without adjustment of diet pH (Cowey, 1994). Results from requirement studies (Gatlin, 1996), comparative studies (Whiteman and Gatlin, 2005) and our own work (Gaylord and Rawles, 2005; Gaylord et al., 2005) suggest that hybrid striped bass respond well to diets containing supplemental amino acids; however, the upper limit of supplementation in these studies was usually below what Shearer (2000), based on the theory of Mercer et al. (1989), would define as inhibiting. In a review by Kasumyan and Døving (2003), Met and Lys were not only categorized as deterrent amino acids in most species investigated, but they were also considered highly aversive taste stimuli, i.e., pellets containing these amino acids were rejected by fish in 100% of trials. Therefore, a maximum to dietary supplementation of Met and Lys may exist, beyond which reductions in palatability and/or metabolic efficiency will occur. If this hypothesis is substantiated, then levels of available Met (2.0 to 2.1 g/100 g diet) and/or Lys (3.9 to 4.4 g/100 g diet) in the replacement diets may have negatively influenced results of the current study. Gaylord and Rawles (2005) included betaine as an attractant in PBM test diets containing supplemental amino acids; however, betaine was not included in these diets in order to limit feed costs. In the future it may be advisable, as other workers have found (Higgs et al., 1995; Kissil et al., 2000), to include palatability enhancers to counter possible depressed intake of replacement diets for hybrid striped bass. It is apparent that additional research is needed regarding the antagonism of feed intake by supplemental amino acids as commercial interest in utilizing novel protein sources increases.

In spite of the less than expected performance of the 70% PBM replacement diet, survival was high (>98%), and weight gains (841% to 709%), final fish weights (825 to 705 g) and total yield (246 to 211 kg) were well within acceptable goals for hybrid striped bass tank production. Given the current prices for fishmeal (>$800/ton; Omega Protein Corp., Hammond, LA, personal communication), petfood-grade poultry by-product meal (≈$360/ton; Tyson Foods, Rogers, AR, personal communication), and feed-grade methionine and lysine ($0.95/lb and $0.74/lb, respectively; ADM Specialty Feed Ingredients Division, Decatur, IL, personal communication), the results of this experiment suggest that poultry by-product meal with the addition of Met and Lys can economically replace at least 35% of the fishmeal in commercial hybrid striped bass diets when substituted on a digestible protein basis.

Acknowledgements

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